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Mechanism of Protein Kinase CK2 Association With Nuclear Matrix: Role of Disulfide Bond Formation

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Abstract Nuclear matrix (NM) appears to be an intranuclear locale for significant and dynamic association of the ubiquitous multifunctional messenger-independent serine/threonine protein kinase CK2 that has been implicated in growth control [Tawfic et al. (1996): J Cell Biochem 61:165–171]. We have examined the nature of the association of CK2 with the NM. Nuclei prepared in the presence of a sulfhydryl-blocking reagent such as iodoacetamide demonstrate a reduction in the amount of CK2 associated with the NM to less than 5% of the control. On the other hand, when nuclei are treated with the sulfhydryl crosslinking reagent sodium tetrathionate, NM-associated CK2 increases severalfold. Treatment of nuclei with sodium tetrathionate followed by 2-mercaptoethanol blocks this increase. Nuclei isolated from rat liver and prostate behaved similarly, suggesting an identical mode of association of CK2 with the NM regardless of the organ. These results indicate a role of sulfhydryl interactions such that NM anchoring of CK2 occurs via its β subunit, which contains several vicinal cysteine residues. Further, various sulfhydryl-blocking reagents inhibited CK2 activity in a concentration-dependent manner, and the inhibitory effect was reversed by agents such as dithiothreitol, implying that cysteine residues in the CK2 play a role in its catalytic activity. J. Cell. Biochem. 69:211–220, 1998.

Key words: nuclear matrix; protein kinase CK2; disulfide bonds; sodium tetrathionate; iodoacetamide; sulfhydryl crosslinking

Nuclear matrix (NM) is the fibrillar network that provides the internal scaffold for the nuclear architecture. Much evidence suggests that NM is involved in the organization of chromatin, thereby playing a fundamental role in many growth-related activities, including DNA replication and control of gene activity in a cell- and tissue-specific manner [Berezney, 1991; Getzenberg et al., 1991; Nickerson and Penman, 1992; Stein et al., 1996]. Isolated NM demonstrates variable and non-variable proteins depending on the nature and state of the cell [Berezney, 1991]. One of the variable NM-associated signaling proteins is the protein kinase CK2, which is dynamically regulated in the NM, as indicated by its modulation in response to growth signals [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996].

CK2 (formerly known as casein kinase 2 or II) is a messenger-independent serine/threonine protein kinase that has been implicated in a variety of cell functions, including growth control. The enzyme consists of α, α′, and β subunits, which may exist in an $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$ configuration in different tissues. The β subunit serves a regulatory role such that it is required for maximal activity of the catalytic α subunit [for reviews, see, e.g., Pinna, 1990; Tuazon and Traugh, 1991; Issinger, 1993; Litchfield and Lüscher, 1993; Ahmed, 1994; Allende and Allende, 1995]. The two β subunits interact, while the two α subunits interact individually with the two β subunits [Litchfield et al.,

Abbreviations: CK2, casein kinase 2; DTT, dithiothreitol; IAA, iodoacetamide; NaTT, sodium tetrathionate; NEM, N-ethylmaleimide; NM, nuclear matrix; PAGE, polyacrylamide gel electrophoresis; p-CMPSA, p-chloromercuriphenylsulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.

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1996; Boldyreff et al., 1996]. The kinase localizes to the cytoplasmic and nuclear compartments where its putative substrates reside, suggesting its involvement in multiple functions [for reviews, see, e.g., Pinna, 1990; Tuazon and Traugh, 1991; Issinger, 1993; Allende and Allende, 1995; Ahmed, 1994; Litchfield and Lüscher, 1993]. Within the nucleus, it appears to associate preferentially with chromatin and NM, suggesting spatiotemporal regulation of its activity [Ahmed et al., 1993; Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996]. For example, the amount of CK2 in rat ventral prostate NM is altered depending on the androgenic status of the animal [Tawfic and Ahmed, 1994a,b]. This finding accords with the androgenic regulation of growth in this accessory sex organ [Williams-Ashman, 1979; Coffey, 1988; Liao et al., 1989]. Thus, the observation on the dynamic association of CK2 with NM depending on the nature of the stimulus is particularly germane to the functional activity of the NM, which may be modulated by the extent of phosphorylation of the NM-associated proteins.

The nature of the association of CK2 with the NM is not known. Recent work utilizing an agent to stabilize the disulfide bonds has suggested that the association of certain proteins with the NM involves formation of disulfide bonds [Stuurman et al., 1992]. Also, a number of the NM-associated molecules such as topoisomerase II [Kaufmann and Shaper, 1991], poly-(ADP-ribose) polymerase [Kaufmann et al., 1991], and glucocorticoid receptor [Kaufmann et al., 1986] appear to involve disulfide bond formation. The α subunit of CK2 contains only two cysteine residues (amino acids 147 and 220); however, the β subunit contains several apparently vicinal cysteine residues (amino acids: 14 and 23; 109 and 114; 137 and 140), raising the possibility of their involvement in interaction with the NM. Based on various observations reported here, we propose that disulfide bond formation is involved in the association of CK2 with the NM. Further, our results suggest that thiol groups in the β subunit play a role in maintaining the activity of the holoenzyme.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 295–325 g (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used as the sources of liver and ventral prostate tissues. The animals were maintained as described previously [Tawfic and Ahmed, 1994a].

Plasmids and Bacteria

Plasmid pBB-3 and pBB-4 containing the human CK2 α and β subunit cDNAs, respectively, and *Escherichia coli* strain BL21 (DE3) were employed for generation of recombinant CK2 as described previously [Grankowski et al., 1991].

Chemicals

Rabbit anti-chicken IgG alkaline phosphatase conjugate was purchased from ICN Biochemicals (Irvine, CA). Synthetic peptide substrate Arg-Arg-Arg-Ala-Asp-Asp-Ser-Asp-Asp-Asp-Asp-Asp was purchased from Peptide Technologies, Inc. (Gaithersburg, MD) and employed for assay of CK2 activity as described previously [Marin et al., 1994]. The following reagents were purchased from Sigma Chemical (St. Louis, MO): sodium tetrathionate (NaTT), p-chloromercuriphenylsulfonic acid (p-CMPSA), methyl methane thiosulfonate (MMTS), iodoacetamide (IAA), and N-ethylmaleimide (NEM). All chemicals used were of the highest purity available.

PREPARATIVE METHODS

Preparation of nuclei and nuclear matrix. Nuclei were isolated from rat liver or ventral prostate as described previously [Tawfic and Ahmed, 1994a,b), and utilized for the preparation of NM according to the procedure described previously [Kaufmann and Shaper, 1991]. The same amounts of nuclei (based on DNA content) were used for isolation of NM following different treatments. In experiments where IAA was utilized, it was present at a concentration of 10 mM in all buffers. When isolated nuclei were treated with IAA, they were incubated with 10 mM IAA for 1 h at 4°C. In experiments where the sulfhydryl crosslinking reagent NaTT was used, nuclei were isolated in the absence of IAA and NaTT and then incubated with 2 mM NaTT for 1 h at 4°C. After incubation with DNase I (250 μg/ml) and RNase A (250 μg/ml) for 1 h at 4°C, nuclei were sedimented and extracted twice with 1.6 M NaCl. Residual material (i.e., NM) was recovered by sedimentation at 5,000*g* for 20 min and solubilized in SDS-PAGE sample buffer.

Preparation and purification of recombinant CK2. Recombinant CK2 was prepared by in vitro self-assembly as described previously [Grankowski et al., 1991; Boldyreff et al., 1993] with minor modifications. Recombinant human CK2 α and β subunits were expressed in bacteria separately, and then the bacteria were mixed in 1:1 ratio. The solutions used for the preparation of the CK2 did not contain dithiothreitol (DTT) but contained 1 mM PMSF and leupeptin (2 μg/ml). The bacterial extract was first subjected to Whatman (Clifton, NJ) P-11 phosphocellulose chromatography. The bound kinase was eluted with a linear gradient of 0.2 M–1.5 M NaCl in TME buffer consisting of 50 mM Tris-HCl buffer, pH 7.9, 1 mM EDTA, and 5 mM $MgCl_2$. The fractions containing active CK2 were pooled and dialyzed overnight against TME buffer containing 0.1 M NaCl at 4°C. The dialyzed material was applied to a DEAE-Sephadex column followed by elution with a linear gradient of 0.1 to 1.0 M NaCl in the same buffer. Active fractions were pooled and dialyzed overnight against TE buffer containing 50 mM Tris-HCl, pH 7.0, 1 mM EDTA, and 0.2 M NaCl, at 4°C. The dialyzed material was run on a spermine-affinity column and eluted with a linear gradient of 0.2–1.5 M NaCl in TE buffer. Fractions containing CK2 activity were pooled and concentrated against TME buffer containing 0.2 M NaCl by using the ProDiCon (Spectrum Medical Industries, Inc., Houston, TX).

The method for preparation of recombinant CK2-α was the same as for the preparation of the holoenzyme described above, with the exception that a heparin-affinity column was substituted for the spermine-affinity column. The CK2-α was eluted from the heparin column with a linear gradient of 0.2 M–1.5 M NaCl in TME buffer.

ASSAYS

CK2 activity. Unless otherwise stated, the general procedure for the measurement of CK2 activity was as described previously [Tawfic et al., 1997]. In brief, the reaction medium contained 150 mM NaCl, 5 mM $MgCl_2$, 50 μM [γ-^{32}P]ATP ($\sim 3 \times 10^6$ dpm/nmol of ATP), 30 mM Tris-HCl buffer, pH 7.45 at 37°C, with and without 0.2 mM peptide substrate, and an appropriate amount of the enzyme protein to give a linear rate of reaction for at least 15 min at 37°C. Tubes without the substrate served as the controls. To terminate the reaction, 40-μl aliquots of the reaction mixture were pipetted onto a 1-inch square of Whatman P-81 phosphocellulose paper. These squares were immediately placed in 10 mM H_3PO_4 at room temperature and washed once for 30 min and once for 15 min in fresh 10 mM H_3PO_4, followed by two 15-min washes in 15% acetic acid. The papers were then washed in acetone for 5 min followed by drying under an air stream. Each paper was placed in a liquid scintillation vial containing 7 ml of the cocktail for spectrometry. Variations from this procedure are indicated in the text.

To assay the kinase activity associated with recombinant CK2-α subunit, the procedure was essentially the same as for assay of the holoenzyme except that NaCl was omitted from the assay medium. To study the effects of the thiol-reactive reagents (IAA, MMTS, NEM, and p-CMPSA) on the activity of the recombinant CK2-α subunit or the recombinant CK2 holoenzyme, the various agents were added to the assay medium used for measurement of kinase activity except that DTT was omitted from the reaction.

Immunodetection of CK2 protein in NM. Protein recovered from an equal amount of DNA in the starting nuclear samples was dissolved by boiling for 5 min in SDS/urea sample buffer containing 8 M urea, 5% 2-mercaptoethanol, and 5% SDS and subjected to electrophoresis on a 10% polyacrylamide slab gel containing 0.1% SDS and 4 M urea. The separated proteins on the gel were transferred to nitrocellulose paper and immunoblotted with an anti-CK2 polyclonal antibody as described previously [Tawfic and Ahmed, 1994a,b].

RESULTS

Involvement of Disulfide Bond Formation in the Interaction of CK2 With the NM

The role of disulfide bond formation in the NM association of certain proteins has been investigated previously by employing NaTT, which promotes disulfide bond formation in cysteine-containing proteins. For example, oxidation of nuclei with NaTT prior to the isolation of NM results in stabilization of the retention of certain proteins via disulfide bond formation [Kaufmann and Shaper, 1991; Kaufmann et al., 1991, 1986; Simon and Pratt, 1995]. We followed the same strategy to determine whether disulfide bond formation played a role in the association of CK2 with the NM. Figure 1 shows

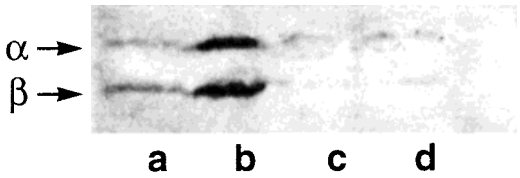

Fig. 1. Effect of sodium tetrathionate and iodoacetamide on the association of CK2 with the nuclear matrix. Nuclei were prepared as described in Materials and Methods and used to isolate the NM after various treatments. The control preparation (**lane a**) was made from nuclei without any further treatment. Purified nuclei were digested with DNase I and RNase A and extracted twice with 1.6 M NaCl. The remaining nuclei were subjected to various treatments as follows. **Lane b:** Purified nuclei were incubated with 2 mM NaTT at 4°C for 1 h and then digested with DNase I and RNase A at 4°C for 1 h. Subsequently, they were extracted twice with 1.6 M NaCl. **Lane c:** The procedure was the same as for lane b except that it included two final extractions with 1.6 M NaCl containing 1% (v/v) 2-mercaptoethanol. **Lane d:** The purified nuclei were incubated with 10 mM IAA at 4°C for 1 h, digested with DNase I and RNase A at 4°C for 1 h, and extracted twice with 1.6 M NaCl. All the nuclease- and salt-insoluble residual structures (i.e., NM preparations) were sedimented at 5,000*g* and solubilized in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol. Amounts of nuclear matrix protein isolated from the same number of starting nuclei (667 μg of DNA) were subject to SDS-PAGE, transferred to nitrocellulose filters, and immunoblotted with anti-CK2 antibody. The arrows indicate the location of the α and β subunits of CK2.

that there was a severalfold increase in immunoreactive CK2 when nuclei were treated with NaTT prior to isolation of the NM (Fig. 1, lane b compared with lane a). Prior treatment of the nuclei with IAA resulted in a significant decrease in the amount of CK2 in the isolated NM (Fig. 1, lane c compared with lane a). Furthermore, when NM was isolated from NaTT-treated nuclei, a subsequent treatment with IAA reversed the enhancement of association of CK2 compared with that observed with NaTT treatment alone (Fig. 1, lane d compared with lanes b and c). These results indicate that association of CK2 with the NM involves disulfide bond formation.

Effect of Thiol-Reactive Agents on the Activity of Purified CK2

The presence of several vicinal sulfhydryl groups in the β subunit of CK2 prompted us to examine the effects of sulfhydryl blocking reagents on the activity of the kinase. These compounds (p-CMPSA, MMTS, IAA, and NEM) inhibited CK2 activity to various degrees in a dose-dependent manner (Table I). The most potent inhibitor was p-CMPSA, which caused 50% inhibition at a concentration of about 20 μM. A similar degree of inhibition was observed in the presence of 2 mM MMTS, 20 mM NEM, or 50 mM IAA. A time course analysis of the inhibition in the presence of these chemical agents at I_{50} concentrations (i.e., the concentration that yielded about 50% inhibition) is shown in Figure 2. Maximal inhibition was achieved within 20–30 min for p-CMPSA, MMTS, NEM, but not for IAA, suggesting that in vitro interaction of the latter with purified CK2 was somewhat slower than that of the other sulfhydryl-reactive agents.

TABLE I. Inhibition of Protein Kinase CK2 Activity by Thiol-Reactive Chemicals*

Inhibitor	Concentration (mM)	Relative CK2 activity
None	—	100
p-CMPSA	0.01	69 ± 8
	0.02	44 ± 9
	0.10	6 ± 5
MMTS	1.0	67 ± 21
	2.0	59 ± 20
	5.0	33 ± 9
IAA	10.0	95 ± 6
	20.0	85 ± 10
	50.0	76 ± 6
NEM	10.0	87 ± 3
	20.0	54 ± 5
	50.0	18 ± 4

*Recombinant CK2 holoenzyme was purified in media without added DTT. It was incubated with various thiol-reactive agents at varying concentrations in a standard reaction medium for assaying CK2 activity, with the exception that no DTT was added. The reaction was carried out for 30 min at 37°C. The data are based on six experiments and are expressed as means ± S.E.M., based on the activity of the control set at 100.

Effect of Dithiothreitol on the Inhibition of CK2 Activity by Thiol-Reactive Agents

Experiments were undertaken to examine the effect of the sulfhydryl-protective agent DTT on the inhibitory effect of the various thiol-modifying agents. The enzyme was treated with the inhibitors for 10 min prior to the addition of 3 mM DTT to the reaction, which was then followed by measurement of the enzyme activity. Under these conditions, DTT reversed the inhibition of CK2 activity evoked by the various thiol-reactive reagents (Fig. 3). Similar results were obtained when 8 mM DTT was used as the reducing agent (not shown). (IAA was not included in these experiments since we observed

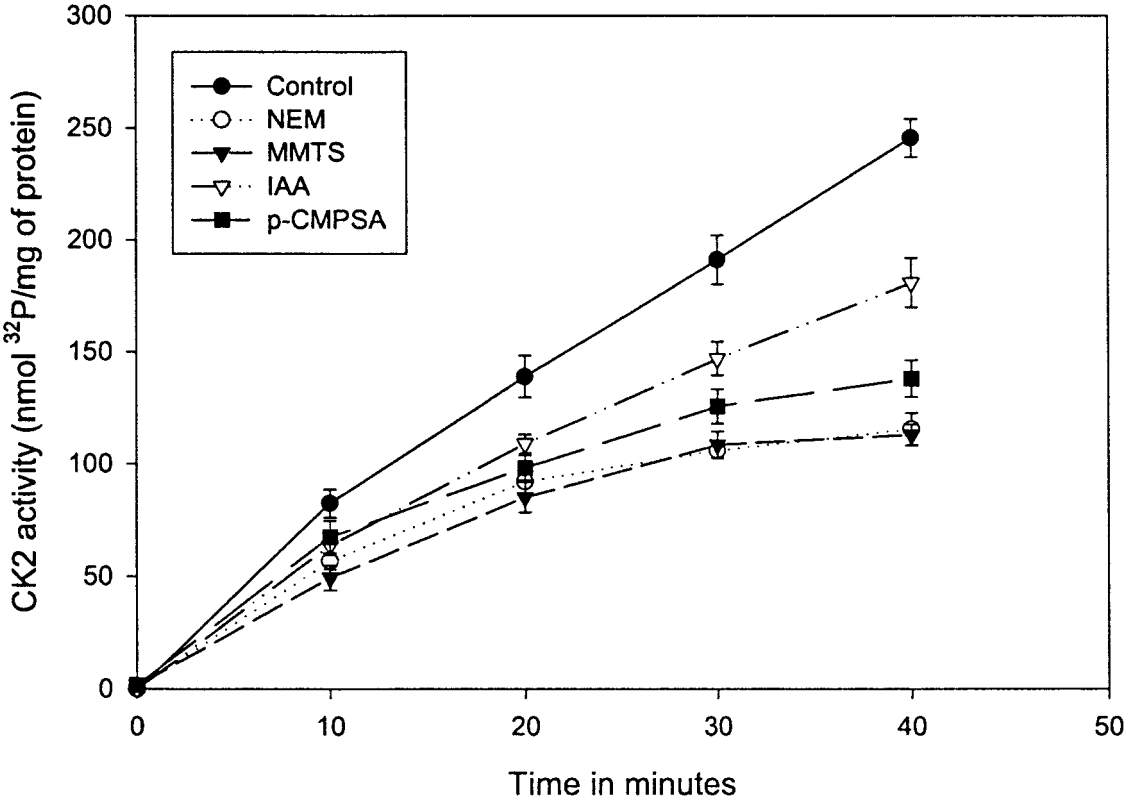

Fig. 2. Time course of the action of various sulfhydryl-reactive reagents on CK2 activity. Purified CK2 (1.5 μg) was treated with 25 mM NEM (○), 3 mM MMTS (▼), 50 mM IAA (▽), 20 μM p-CMPSA (■), or no addition control (●) in the standard reaction medium (final volume 300 μl) except that DTT was not included. The protein kinase reaction was started by the addition of [γ-^{32}P]ATP and continued for the times indicated.

that addition of DTT resulted in the release of iodide, which inhibits the enzyme.)

Effects of Thiol-Reactive Agents and Dithiothreitol on the Kinase Activity of Recombinant CK2-α Subunit

Since CK2-α contains two cysteine residues and also exhibits catalytic activity in the absence of the β subunit [Grankowski et al., 1991], we decided to examine the effects of the various thiol-reactive agents on its enzyme activity. The CK2-α was preincubated with or without thiol-reactive agents prior to the addition of 8 mM DTT, which was then followed by measurement of the enzyme activity. The results in Figure 4 show that most of the thiol-reactive agents had a minimal effect on the enzyme activity, and the only agent that had some inhibitory effect was MMTS at a concentration of 1 mM. Also, in this assay, the addition of DTT had no effect on the catalytic activity associated with α subunit. The activity of a representative preparation of CK2-α was 2,100 nmol ^{32}P/mg of protein/h in the absence or presence of 8 mM DTT. In contrast to this result, the addition of DTT to the kinase assay of the recombinant CK2 holoenzyme gave a concentration-dependent stimulation of the activity, reaching a maximal of 50% in the presence of 8 mM DTT (Table II). Taken together, these results suggest that the sulfhydryl groups in the β subunit of CK2 play a role in maintaining CK2 activity.

DISCUSSION

NaTT has been employed previously as the thiol-oxidizing reagent to show that sulfhydryl bond formation via cysteine residues is involved in the NM association of a number of intrinsic non-variable proteins as well as certain other variable proteins [Stuurman et al., 1992; Kaufmann and Shaper, 1984]. Among the NM-associated proteins thus investigated were a 38-kDa nucleolar protein [Fields et al., 1986], the glucocorticoid receptor [Kaufmann et al., 1986], a 62-kDa protein present in metaphase chromosomes [Fields and Shaper, 1988], SV-40

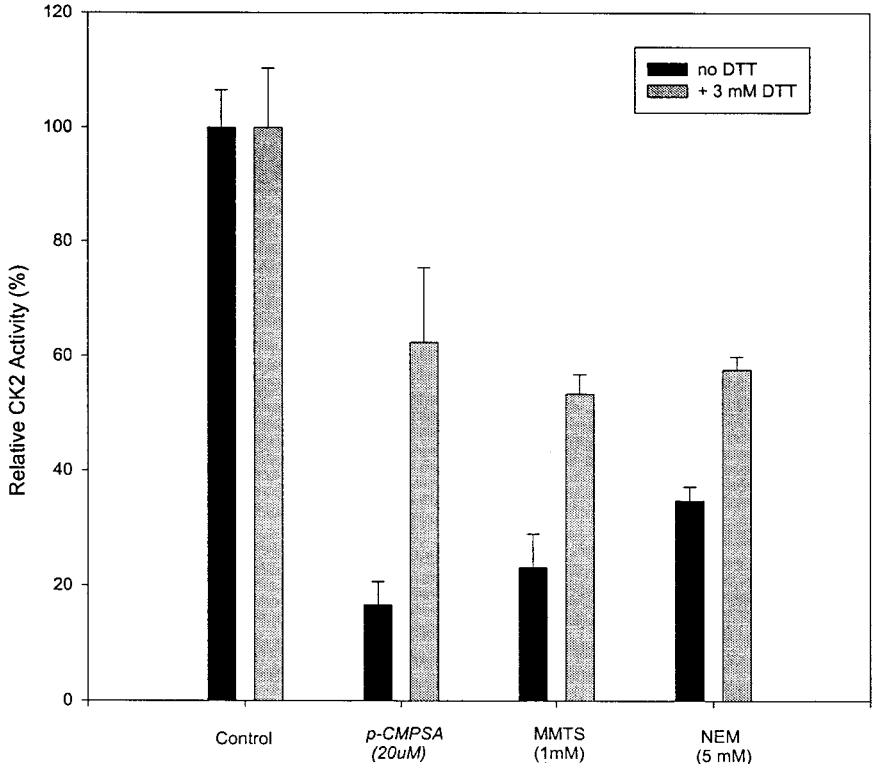

Fig. 3. Effect of DTT on the inhibition of CK2 in the presence of various thiol-reactive agents. Purified recombinant CK2 was suspended in 30 mM Tris-HCl buffer (pH 7.45) and treated with various thiol-reactive agents at the indicated concentrations for 10 min at 37°C. DTT was then added at a concentration of 3 mM followed by addition of [γ-^{32}P]ATP to initiate the kinase reaction. The reaction time was 30 min at 37°C. The results are expressed as percent of the control values and are presented as mean ± S.E.M.

large T antigen [Humphrey and Pigiet, 1987], topoisomerase II [Kaufmann and Shaper, 1991], and poly(ADP)-ribose polymerase [Kaufmann et al., 1991]. In all these cases, retention of the polypeptides was found to be enhanced by disulfide bond formation induced in vitro by the action of NaTT, a process that is inhibited by agents such as IAA or reversed by DTT or 2-mercaptoethanol. It has also been established that proteins in the NM are stabilized by tetrathionate via the oxidation of sulfhydryls to disulfide bridges [Kaufmann and Shaper, 1984] and that a significant number of the polypeptides in the NM undergo disulfide bond formation under these conditions [Stuurman et al., 1992].

The work presented here has employed the same strategies to determine if similar interactions are involved in CK2 association with the NM, and the results suggest that the association of CK2 may be via the β subunit as it contains several vicinal cysteine residues that may interact with similar residues in the binding sites in the NM. Further, because two of the β subunits in CK2 are linked [Litchfield et al., 1996; Boldyreff et al., 1996], it would appear that the number of sulfhydryl groups that might be available for binding to the NM through the disulfide linkages would be doubled. However, the involvement also of the two cysteine residues in the α subunit cannot be ruled out.

In previous work, we provided evidence that a significant amount of CK2 in the nucleus is associated with the isolated NM. This association appears to be physiologically relevant in the phosphorylation of proteins intrinsic to the NM and is dynamically regulated by growth signalling [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996]. Further, the presence of CK2 in the isolated NM can be shown by employing different isolation procedures [Tawfic et al., 1997]. Thus, the association of CK2 with the NM appears to be analogous to that of the aforementioned peptides such as the glucocorticoid receptor, topoisomerase II, and poly(ADP)-ribose polymerase [Kaufmann and Shaper, 1991; Kaufmann et al., 1991, 1986]. Considerable evidence exists to support the notion that

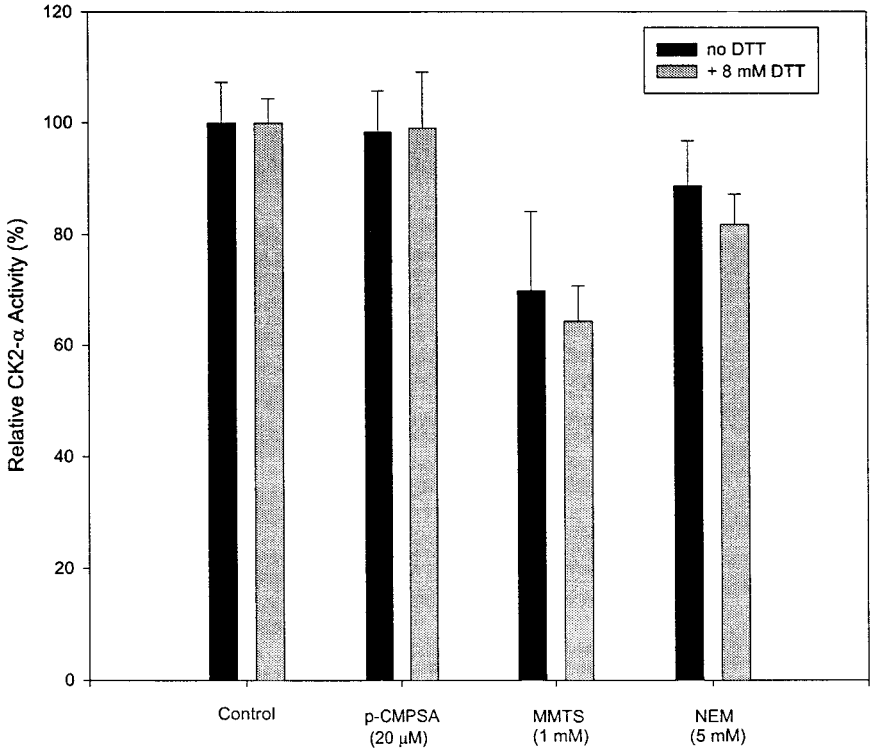

Fig. 4. Effects of sulfhydryl-reactive agents and dithiothreitol on the recombinant CK2-α subunit kinase activity. Purified recombinant CK2-α subunit was suspended in 30 mM Tris-HCl buffer (pH 7.45) containing 5 mM $MgCl_2$, and treated with various thiol-reactive agents for 15 min at 24°C. This was followed by addition of 8 mM DTT, and the kinase reaction was initiated by addition of [γ-^{32}P]ATP. The reaction time was 15 min at 37°C. Results are expressed as percent of control, and are presented as mean ± S.E.M.

TABLE II. Effect of Varied Concentration of DTT on the Activity of Recombinant Protein Kinase CK2*

DTT (mM)	CK2 activity	Percent change
None	3,143 ± 162	—
0.5	3,422 ± 91	9
1.0	3,655 ± 164	16
2.0	3,894 ± 182	24
3.0	4,283 ± 185	37
4.0	4,492 ± 154	43
6.0	4,681 ± 73	49
8.0	4,754 ± 131	52

*Recombinant CK2 holoenzyme was purified in media without added DTT. Varying concentrations of DTT were added in a standard reaction medium for CK2 assay. The reaction was carried out for 20 min at 37°C. The CK2 activity based on six experiments is expressed as mean ± S.E.M., and is defined as nmol ^{32}P/mg protein/h.

NM interactions of the various NM binding proteins are not artifacts induced by random sulfhydryl crosslinking by NaTT. For example, it has been documented that NaTT stabilization of isolated nuclei does not alter the spatial distribution of some of the NM proteins examined [Neri et al., 1995]. Specificity of NaTT-mediated association of androgen and glucocorticoid receptors with NM has been found to relate to specific domains in these molecules. Mutation of a single cysteine in the DNA binding domain of the glucocorticoid receptor resulted in a dramatic reduction in receptor binding to NaTT-stabilized nuclei and NM. The view that NM association of proteins involves certain specificity is further reinforced from studies on a fusion protein consisting of β-galactosidase linked to the nuclear localization signal of SV40 large T antigen. This protein readily enters the nucleus, but, despite the presence of 16 cysteine residues as potential targets of NaTT, was found to show no detectable binding to the nuclei or NM with or without NaTT treatment [Van Steensel et al., 1995].

Reversible disulfide bond formation may be important in the spatiotemporal anchoring of CK2 to the NM. However, it is not clear if disulfide bonds form between CK2 and NM protein(s) in vivo. The enzymes that have been implicated in the regulation of the oxidative

state of thiols are glutathione S-transferase [Bennett et al., 1986], thioredoxin, and thioredoxin reductase [Rozell et al., 1988]. These enzymes are located in the nucleus and may be active in the regulation of several factors involved in signal transduction such as the NM-associated glucocorticoid receptor [Kaufmann et al., 1986; Simon and Pratt, 1995]. The significance of disulfide interactions is becoming increasingly apparent [Ziegler, 1985; Holmgren, 1989]. In particular, it has been proposed that vicinal sulfhydryls have specific functions in the NM [e.g., Stuurman et al., 1992; Kaufmann and Shaper, 1984]. In this regard, it may be noteworthy that a 57-kDa protein with thiol: protein-disulfide oxidoreductase activity is localized in the NM [Altieri et al., 1993]. Previous studies on disulfide bond formation in the NM have suggested that many proteins participate to some extent in the formation of disulfide bridges. It was observed that most of the NM-associated polypeptides that contain oxidized sulfhydryl groups (i.e., in response to NaTT) actually form disulfide bonds in the NM [Stuurman et al., 1992]. We have not determined which of the proteins in the NM participate in the formation of disulfide bonds with the CK2. Further, the present work does not reveal whether one or more of the six cysteine residues (i.e., cysteines 14, 23, 109, 114, 137, 140) in the β subunit of CK2 participate in disulfide bond formation with the NM, although it is very likely that the cluster of vicinal cysteine residues 109, 114, 137, 140 plays a primary role. A model showing these interactions of CK2 with the NM is proposed in Figure 5.

In the present work, we have also shown that purified preparations of recombinant CK2 require a sulfhydryl-protective agent for apparent maximal enzyme activity. A systematic analysis of the role of sulfhydryls in CK2 activity has not been undertaken previously. Several of the sulfhydryl-blocking agents inhibited the enzyme to various degrees but the sensitivity to the sulfhydryl-blocking agents was moderate, and this inhibition was only partially reversed by sulfhydryl-protective agents such as dithiothreitol even at a concentration of 8 mM in the reaction. However, our results have established that the inhibition of the CK2 holoenzyme activity is not due to effects of the thiol-reactive agents on the two cysteine residues in the α-subunit of the enzyme since the catalytic activity associated with the CK2-α subunit was not

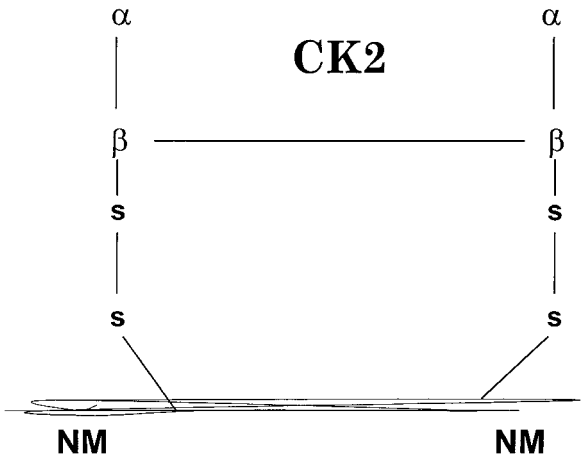

Fig. 5. A proposed model depicting the interactions of protein kinase CK2 with the NM. Based on the current proposals, the two α subunits (or α and α′) are shown to be linked to the two β subunits independently while the two β subunits interact with each other. The sulfhydryl groups in the two β subunits are proposed to interact with the sulfhydryl groups of the NM proteins, which might serve as the acceptors for the binding of CK2 to the NM.

inhibited by these agents. This suggests that the inhibitory effects of these agents are mediated primarily via their interaction with the cysteines of the β subunit of CK2, although it is not clear which of the six cysteines in the β subunit are directly affected by these agents. It may be speculated that the action of sulfhydryl-blocking agents is related to subunit interaction, thus reflecting on the total enzyme activity. In this regard, it is noteworthy that the stimulatory effect of DTT on the kinase activity is apparent only with the holoenzyme and not with the activity of the α-subunit alone, again suggesting the involvement of the interaction of the cysteine residues in this phenomenon. Further, the partial reversal of the inhibitory effects of thiol-reactive agents on CK2 by DTT suggests that some of the thiol(s) in the CK2 may function through disulfide bond formation in addition to some functional activity of free sulfhydryl group(s). In such a case, it is plausible that besides being a protective agent DTT may also exert additional effects on the kinase activity. This possibility accords with the reports based on functional studies employing discrete mutants of the α and β subunits of CK2. For example, the requirement of residues 132–165 for the dimerization of the β subunits in the CK2 holoenzyme [Kusk et al., 1998] may involve disulfide bond formation through cysteines 137 and 140. It has also been suggested

that the minimal fragment of the β subunit that may allow dimerization appears to range between amino acids 20 to 165 [Kusk et al., 1995]. This would imply that in addition to 137 and 140, other cysteines (i.e., cysteines 23, 109, 114) could conceivably also be involved in disulfide bond formation.

In summary, the present work has demonstrated, for the first time, that association of protein kinase CK2 with the NM is enhanced by an agent that stabilizes disulfide bonds, whereas it is reduced by agents that block sulfhydryl groups. Thus, disulfide bond formation may play a significant role in the association of CK2 with the NM. Further, various data indicate that the cysteine residues in the β subunit of the enzyme play a significant role in the structure/activity of the enzyme.

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